Effects of fungicides on a Fusarium sp. biological control agent of Botrytis cinerea stem infections in the perspective of an integrated management of fungal diseases in greenhouse tomatoes

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Effects of fungicides on a *Fusarium* sp. biological control agent of *Botrytis cinerea* stem infections in the perspective of an integrated management of fungal diseases in greenhouse tomatoes

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Abstract: Hexaconazole and difenoconazole have a strong effect on radial and germ tube growth of the *Fusarium* sp. biocontrol agent across the all tested concentrations. The active ingredients (vinchlozolin, bupirimate, pyrimethanil) and the mixtures of cymoxanil + mancozeb and diethofencarb + carbendazim have little or moderate effect *in vitro*. *Fusarium* sp. strain was still efficient to protect the pruning wounds against *B. cinerea* and survived well on the plants treated with these active ingredients. This biological *Fusarium* sp. strain could be used in alternation or in combination with these fungicides in an integrated management of gray mold and fungal diseases in tomatoes greenhouse.

Key words: gray mold, biological control, chemical control, integrated control

Introduction

Suppression of stem infections by *B. cinerea* can be achieved by an integrated approach combining chemical treatments with other control measures (Elad *et al.,*1995; Nicot & Baille, 1996). However, disease management by fungicides is complicated by the development of resistant strains. Integration of fungicides with biocontrol agents could provide an opportunity to reduce the pressure of fungicides on the pathogen and so the risk of development of resistant strains. This alternation would also reduce pesticide residues in foods. Moreover, the alternation or combination of fungicides with antagonists may provide better control than the antagonist alone (Elad *et al*., 1996).

Recently, we isolated a fungal antagonist, provisionally identified as *Fusarium* sp., for its efficiency to protect the pruning wounds on tomato plants (Nicot *et al*., 1996; Decognet *et al*., 1997). This paper examines the effects *in vitro* and *in vivo* of fungicides on *Fusarium* sp. in order to combine biological and chemical methods to control *B. cinerea* stem infections in an integrated management of fungal diseases in tomato greenhouses.

Material and methods

The sensitivity and efficiency of *Fusarium* sp. was tested against active ingredients registered in France for use on tomato to control *B. cinerea* (vinchlozolin, pyrimethanil, dichlofluanid, diethofencarb + carbendazim), *Alternaria* spp. (difenoconazole), *Oïdium lycopersicum* (bupirimate, hexaconazole), *Phytophthora infestans* (cymoxanil + mancozeb, dichlofluanid).

Sensitivity of Fusarium sp. to fungicides

The commercial formulations were added to molten Potato Dextrose Agar (PDA), except for pyrimethanil for which the medium described by Leroux & Gredt (1996) was chosen. The tested concentrations of active ingredients are indicated in table 1a and 1b. To assess the effect on the hyphal growth, Petri dishes were inoculated in the centre with a 4,5-mm diameter disk from a 13 days-old colony of *Fusarium* sp. After seven days of incubation at 21°C, the diameter of the colony was measured to express the percent of reduction in growth compared to the untreated control.

The sensitivity of the conidia was tested by plating 0,1 ml of a 5 x 10^3 conidia suspension per ml over the surface of the media. Elongation of the germ tube of 60 spores was determined by microscopic examination after 24 hours of incubation at 21°C and the effect of the fungicide was expressed by the reduction of germ tube growth compared to the untreated control.

Effect of fungicides on the efficiency and survival of Fusarium sp.

Shake cultures of the fungal antagonist were prepared in Yeast-Malt extract broth. Prior to utilisation, the cell suspensions were filtered to remove mycelium fragments, centrifuged and resuspended in water.

Spores of *B. cinerea* were obtained after 10 days of incubation on PDA by washing the cultures with sterile water. To obtain development of gray mold on pruning wounds treated with botryticides, resistant strains of *B. cinerea* were chosen. The strain BC1 (isolated from tomato) resistant to vinchlozolin was inoculated on plants treated with this active ingredient or with hexaconazole, difenoconazole, bupirimate or the mixture of cymoxanil + mancozeb. Strains resistant to dichlofluanid (SAR 3182) and to diethofencarb + carbendazim (SAR 11092) were provided by Dr F. Faretra. A strain resistant to pyrimethanil (28xsp, isolated from grapevine) was supplied by Dr P. Leroux.

Leaves on 8-week old tomato plants, cv "*Monalbo,* were removed by cutting the petioles at 5-cm from the stem. The wounds were burned before depositing *B. cinerea* and the antagonist as drops of 10 ml of spore suspension (10^4 spores per wound of each fungus). The commercial formulations of fungicides were sprayed one hour later at the rates used in greenhouse tomatoes (Table 2). Plants were incubated in a growth chamber under a plastic film to promote *Botrytis* infection. The progression of lesions on the petiole stubs was measured daily. The areas under the disease progress curves (AUDPC) were used to calculate the percentage of protection provided by the antagonist (Nicot *et al*., 1993).

The survival of the *Fusarium* sp. was evaluated after 14 days by grounding petiole stubs in phosphate buffer with an Ultra-Turrax (20500 tours/min). Serial dilutions were plated on peptone-PCNB-agar medium (Nelson *et al*., 1983). The results were expressed as colony forming units (CFU) per gram of fresh tissue.

Results

Sensitivity in vitro of the Fusarium sp. strain *to fungicides*

Among all tested active ingredients, the *Fusarium* sp. strain was most sensitive to difenoconazole (Table 1). The percentage of inhibition of the radial growth and of the germ tube elongation was superior to 80% at the lowest tested dose, 5 mg/l. Elongation of the germ tube was completely inhibited by concentrations above 25 mg/l. The *Fusarium* strain was also strongly affected by hexaconazole. This active ingredient reduced both radial and germ tube growth at 5 mg/l by 59,7 and 47,2%. Percentages of inhibition above 80% were observed for concentrations at 50 and 100 mg/l. Lower sensitivity to dichlofluanid was observed. Radial growth was slightly affected by concentrations lower than 10 mg/l and reduced by 80% at 50 mg/l. However, elongation of the germ tube was strongly inhibited at 10 mg/l (90,1%) and completely inhibited for the highest tested concentrations. Vinchlozolin inhibited moderately the radial growth (27,2 to 48,2%) and germ tube elongation (65,0 to 73,9%). The *Fusarium* sp. was little sensitive to bupirimate. The highest percentage of inhibition was observed for the radial growth which was reduced by 51,2% for a concentration of bupirimate of 100 mg/l. Pyrimethanil and the mixture of diethofencarb + carbendazim had a slight effect on radial growth (0-16,7% of inhibition) and germ tube elongation (0-37,4% of inhibition) (Table 1a and 1b). Radial growth was little affected by the mixture of cymoxanil + mancozeb for the concentrations of the fungicide (Remiltine pepite) below 100 mg/l (up to 47,1%) and completely inhibited for a concentration at 200 mg/l (Table 1b). An inhibition of the germ tube elongation superior to 80% was measured whatever the tested concentration of Remiltine pepite.

Effect of fungicides on the efficiency and survival of Fusarium sp.

The progression of *B. cinerea* was slowed or completely inhibited by the antagonist applied on petiole stubs of plants not treated with fungicides (Table 2). High rates of protection (67 to 100%) were provided according of the *B. cinerea* strain inoculated on these petiole stubs.

The protection afforded by the botryticides was moderate (diethofencarb + carbendazim), low (vinchlozolin) or null (dichlofluanid) due to the inoculation with strains of *B. cinerea* resistant to these active ingredients (Table 2). At the opposite, no infection of the pruning wounds was recorded on plants treated with pyrimethanil although the strain of *B. cinerea* inoculated was resistant *in vitro*. When the antagonist was applied on pruning wounds of plants sprayed with water or pyrimethanil, 100% protection was observed. The application of *Fusarium* sp. on plants treated with diethofencarb + carbendazim, vinchlozolin, dichlofluanid allowed to obtain high rates of protection (82 to 100%). For vinchlozolin, synergism may have occurred because the protection provided by *Fusarium* sp. on plants treated with vinchlozolin was higher (89%) that on the petiole stubs protected by the antagonist alone (67%) or by vinchlozolin alone (12%).

The four other active ingredients tested are not registered botryticides. However, hexaconazole and difenoconazole provided a moderate protection against infection by *B. cinerea* (about 50%). High rates of efficiency were observed when the antagonist was combined with a spray of these active ingredients (about 90%). Bupirimate and cymoxanil + mancozeb failed to inhibit the development of *B. cinerea*. The level of protection provided by the antagonist was similar on plants treated with bupirimate than on plants not treated. At the opposite, the spray of cymoxanil + mancozeb reduced the efficacy of the antagonist from 67% to 34%.

The population of *Fusarium* sp. was monitored 14 days after its application in petiole stubs. No or limited changes were observed between the population size in petiole stubs on plants treated with fungicides or not (Table 2)

Conclusion

Although effects of fungicides were observed on agar tests, the efficiency of *Fusarium* sp. to protect the pruning wounds on tomato plants was not or only moderately affected by the fungicide treatment. Moreover, the application of the antagonist allowed restoring a high protection when the efficacy of the botryticides was reduced because of the use of resistant strains. These results are very promising because this biological agent could be used in alternation or combination with fungicides in an integrated management of fungal diseases on tomato crops. These results are also interesting in the perspective of a development of this biocontrol agent in viticulture because all tested active ingredients (except bupirimate) are used on this crop.

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Tables 1: Effect of active ingredients (Table 1a) and mixtures of active ingredients (Table 1b) on radial growth and germ tube elongation of conidia of *Fusarium* sp antagonist of *B. cinerea.*

Table 1a

Active ingredient (Fungicide)	% inhibition of the radial growth					% inhibition of the germ tube growth				
	Concentration of active ingredient (mg/l)									
	5	10	25	50	100	5	10	25	50	100
hexaconazole (Anvil)	59,7	67,8	79,5	81,6	80,9	45,2	52,9	73,2	80,3	80,0
difenoconazole (Score)	80,9	83,6	88,1	89,1	90,4	95,5	97,5	100,0	100,0	100,0
dichlofluanid (Euparene)	11,3	17,3	66,1	88,3	91,5	13,2	90,1	100,0	100,0	100,0
vinchlozolin (Ronilan DF)	27,2	48,4	45,9	47,3	39,9	67,7	73,9	71,7	70,9	65,0
bupirimate (Nimrod)	3,9	10,2	20,8	31,1	51,2	0	0	17,8	34,7	44,6
pyrimethanil (Scala)	0	4,2	*	14,6	18,8	2,5	0	*	0	37,4

*: not tested

Table 1b

% inhibition	Concentration of fungicide (mg/l)									
	diethofencarb + carbendazim (Sumico)					cymoxanil + mancozeb (Remiltine pepite)				
	0,08	0,2	0,4	1	2	10	25	50	100	200
radial growth	17,3	12,0	11,3	15,2	14,8	9,6	21,2	31,1	47,1	100,0
germ tube growth	19,2	34,4	26,5	22,5	18,9	80,2	100,0	100,0	100,0	100,0

Table 2: Effect of fungicides on efficiency of *Fusarium* sp. to protect the petiole stubs on tomato plants against infection by *B. cinerea* and on its survival in these petiole stubs.

Active ingredient (Fungicide)	Field rate (mg/l) *	Percentage of protection			Log_{10} (CFU/g tissue)		
		Fusarium alone	Fungicide	Fusarium + Fungicide	Fusarium alone	+ Fungicide	
hexaconazole (Anvil)[a]	30	67	45	98	6,3	6,4	
difenoconazole (Score)[a]	125	67	49	92	6,3	6,1	
vinchlozolin (Ronilan DF)[a]	750	67	12	89	6,3	6,4	
bupirimate (Nimrod) [a]	500	67	- 5	68	6,3	6,2	
cymoxanil + mancozeb (Remiltine pepite)[a]	2500	67	- 1	34	6,3		6,2
dichlofluanid (Euparene)[b]	1250	100	-12	82	5,3	5,6	
diethofencarb + carbendazim (Sumico L)[c]	2	100	46	100	6,3		6,4
pyrimethanil (Scala)[d]	800	100	100	100	6,1	5,5	

*: Field rate concentration of active ingredient or fungicide (for mixture of active ingredients) assuming a spray volume of 1000 l/ha;

a, b, c, d: plants inoculated with BC1 strain of *B. cinerea* resistant to vinchlozolin (a); with SAR 3182 resistant to dichlofluanid (b); with SAR 11092 resistant to diethofencarb + carbendazim (c); with 28xsp resistant to pyrimethanil (d)